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May-21-2004 01:50pm From- T-092 P.002/008 F-8

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Robert Michael ROBERTS Ex Jonathan Andrew GREEN and Sancai XIE

Serial No.: 09/273,164

Filed: March 19, 1999

For: COMPOSITIONS AND METHODS FOR EARLY PREGNANCY DIAGNOSIS

Group Art Unit:

Examiner:

1643

C. Chen

Atty. Dkt. No.: UVMO:003

CERTIFICATE OF MAILING 37 C.F.R. 1.8

3/(1.1.6

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Med in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below:

Date

Robert E. Hanson

DECLARATION OF JONATHAN A. GREEN UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents Washington, D.C. 20231

L JONATHAN A. GREEN, HEREBY DECLARE AS FOLLOWS:

- 1. I am a co-inventor of the subject matter disclosed and claimed in the above-referenced patent application.
- 2. I am currently employed by The University of Missouri as an Assistant Professor. I hold a Ph.D. in Biochemistry from the University of Missouri. I have been conducting research in the area of biochemistry and reproductive biology since 1991.

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- I previously supplied a Declaration in this patent application submitting data demonstrating the isolation and use of monoclonal antibodies that detect PAGs disclosed in the above-referenced patent application. The data presented at that time demonstrated that PAGs 4, 6, 7, 16 and 20 are absent about two-months post-partum and that antibodies for these PAGs may be used in assays for the detection of pregnant bovine animals. I am submitting this Declaration to present further data obtained since the time of the studies described in my previous Declaration. This data demonstrates that, in addition to the PAGs listed above, PAG 17 and PAG 21 are also undetectable at about two months post-partum.
- 4. Identification of PAGs bound by L4, A6 and J2 monoclonal antibodies.

The isolation of monoclonal antibodies I.4, A6, and J2 was as described in my previous Declaration. Further studies were carried out under my supervision to identify the PAGs detected by these antibodies as follows:

One mg of each purified mAb was first crosslinked to 2 mL of matrix in the ImmunoPure Protein A IgG Plus Orientation kit (Pierce Biotechnology, Inc. Rockford, IL, USA) by following the manufacturer's instructions. Cotyledonary extracts were collected from 18 cm and 40 cm crown-nump bovine fetuses, dialyzed against 2000 volumes of binding buffer and 100 mg of total protein from each extract was applied separately to each matrix. The columns were washed in binding buffer until the absorbance of the flow-through at 280 nm was at baseline. The bound protein was eluted from the column with 20mM sodium formate, pH 2.8. The eluted proteins were subjected to SDS-PAGE followed by in-gel trypsin digestion, reduction and alkylation of cysteines. The masses of the resulting peptides were then determined by Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry on a Voyager-DETM PRO Biospectrometry Workstation (Applied Biosystems, Foster City, CA, USA). The monoisotopic masses in the acquired spectra were used for searching against a nonredundant translated mammalian sequence database (NCBlnr) by using the Protein Prospector MS FIT program (http://prospector.ucsf.edu/).

The A6 monoclonal antibody exhibited the greatest ability to bind PAG in the placental extracts. The eluted material migrated at three distinct relative molecular mobilities on SDS-PAGE: 55,000, 65,000 and 75,000. Peptide mass fingerprinting revealed that the 75kDa band

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consisted predominantly of PAG7 and PAG6 and the 55kDa band consisted predominantly of PAG16 and PAG4, although weak matches were also observed for PAG17, PAG20 and PAG21 in this band (Table 1). The 65kDa band did not produce many peptides amenable to fingerprinting, but the few that were produced were found to match PAG7.

The yields from the J2 and L4 affinity columns were not as robust as those from the A6 matrix, however, both did permit the purification of a ~70,0000 Da protein. The J2-purified protein did not produce many tryptic fragments, but those that were produced matched PAG20 (Table 2). The L4-purified protein was more easily digested and produced numerous masses for fingerprinting. The main PAG isolated from the extracts was PAG21 although other PAGs (PAG17, PAG16 and PAG20) were clearly represented, albeit at lower concentrations (Table 3). The A6 and L4 antibodies bound PAG17 with lower affinity, but the results confirmed the undetectability of this PAG at about two-months post-partum. The major PAGs recognized by each of the monoclonal antibodies (and their relationship to other bovine PAGs) are indicated in the neighbor-joining tree (FIG. 1).

- 5. The result of the studies demonstrated that PAGs 4, 6, 7, 16, 17, 20 and 21 are undetectable about two-months post-partum and that antibodies for these PAGs may be used in assays for the detection of pregnant bovine animals.
- 6. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date

May 2004

Jonathan A. Green

Table 1. Assignment of tryptic digest fragments of PAG affithy purified with the AB reproduced anthody.

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	2297;488 7.97 147-467 2300,2138 3.0885 856-887	1878-289 1378-289 1.523 382-372 (977-59-17443-14460) 1488-807 1431.7144 84.5322 113-427 (450-174-174-174) 1782-849 1781-899 46.533 213-247 (450-174-174-174-174) 1771-849 1771-849 -14.1511 1171-827 (750-174-174-174-174-174-174-174-174-174-174	IRUS (AF020000) prograsop-associated at MH+ mat/2564, Delta norm. Bital 520-3442 -4.1081 175-375 833-344 1221-5832 44559 335-344 1221-5832 9.5465 1225-135	268,1292 5,9259 148471) 1674.6917 - 5.1701 209.422 () 259.2281 - 6.1239 - 448-470 () 2541.1772 - 1.7756 - 216-442 (1948.5410 1046.5311 6.4654 350.530 (R]AYSHARQB) 1256.0730 1256.0503 4.69199 157-447 (R]AHRQVAKYDTVR(I) 1254.6170 1256.0152 1,3071 157-447 (R]AHRQVAKYDTVR(I) 1470.6170 1476.7064 8.642 30-42 (R)TLGGGAKLAHVLQGS) 1486.7300 1486.7239 4.3312 322359 (R]AYSHARQBQAKLAHVLQGS) 1522.0530 1622.7338 8.1653 310-422 (R)TLGGWAVLQGSVFLQG)	RUS, (W702050) programs/ estautabel d. 1921 malched. Della peri. Emanuel. 583,3462 4, 1931 505-538 178,4465 47,962 505-53 785,4594 40725 150-538 871,6591 -2,4698 343-549	75mDa protein
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- Wester maid	£191.007 £291.221 2441.292 2507.297 2700.408 9094.052	1251.827 1348.680 1488.680 1622.883 1628.680 1628.680		1738,8290 1959,9220 2012,0830	1622,7788	1388,6428 1405,6410 1422,6550 1430,6750 1480,6880 1674,7878	1. 802.761 002.360 002.360 002.453 000.623 000.623 000.633 000	55 KDa pedelni*
*Weeler middled were observed for PAS-17, PAS-20 and PAS-21	2191.0606 2254.1987 2411.2404 2547.2332 2700.4669 3068.6361	1340,7061 1340,7422 1490,1038 1632,3855 1632,8321 1887,0285 2013,0867	2. BCS TALRUS. (AFDZDEN) programy mic submitted. Mile micched. Edita som mic submitted. Mile mich mich	1759,2942 1859,575 2012,0857	1627,7501 1627,7541	1381,0585 1421,8534 1421,6478 1431,7238 1481,7238 1516,685	1. BUS TALIRUS. (AF1923X1) programo- prit grizminal liefo meta-bet. Dela name 169.3000 129.3462 47.2865 727.4560 727.4465 43.2965 (06.5304 (146.55) 43.7317 1167.5596 1167.591 - 27.4764	
ed for PAG	12,0431 5,0197 8,5788 21,9456 20,9669	-25.0536 -12.4913 -70.2132 -1.6811 -1.267 -1.278	Defautoja 630/82 63,727 -18,9622	0.48H 25.24 -1.3362	02040	-15.657 5.1948 -15.657		
17, PAG-2		######################################	125-125 186-725 187-725 188-72			116-228 218-228 218-228 115-128 116-128	8000mbd 9 8000 8000 974-978 388-388 382-389 342-381	
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Table 2. Assignment of tryptic digest fragments of PAG affinity purified with the J2 monoclassal antihody.

BOS TAURUS.	(AF192337) prog	mancy-66800	delek glycop	rotein-20	
m/z submitted	MH+ matched.	Daite pom	<u> Stert</u>	<u>Posido 3 esvence</u>	<u>Mocifications</u>
1046,580	1046,5311	52,4526	362-369	(R)LYF\$VFDR(G)	
1687.881	1667.6007	0.2052	2 9 42	(R)KTLSGKNMLNNFLK(E)	1P04
1789.976	1789.9271	26.7724	123-138	(R)LTNKTFGITYGSGRMK(G)	1Met-ox
1794.B82	1704.8039	43.5329	217-231	(K)GSYVMFGGVDHRYYK(G)	1604
1895.032	1895.0505	-8.7585	2 -18	(K)WLVLLGLVAF8ECIFK(I)	
1927,919	1927,9264	-3.8439	327-342	(R)AYVLKDFTGNCYTTFR(E)	
****	COOK 4500	60 4707	449 440	CONCOTED THAT BOLL VOCADIM	1



Table 3. Assignment of tryptic digest fragments of PAG affinity purified with the LA monoclonal antibody.

8OS TAURUS. (AF192356) pregnancy-essociated plycoprotein-21.							
m/z sukanitted	MP++ metiched	Delta ptati	Start	Paptide Sequence	Modifications		
642.337	842,8464	12.626	<u> 282-288</u>	(K)LVNKIQK(L)			
965,513	965,542	-30.0409	327-334	(R)AYILKDSR(G)			
87 0. 51 0	970.6413	-23.0012	281-288	(R)KLVNKICK(L)			
1032.578	1012.5155	60,5788	362-369	(R)VYFSVFDR(G)			
1088.598	1088.5376	55.4425	127-136	(K)TFSITYGSGR(M)			
1178,673	1178.6546	7.1482	327-9 96	(R)AYILIOSRGR(C)			
1201.61	1201.6152	- 4.3171	337-345	(R)CYTAFKKQR(F)			
1569.723	1389.0505	46,4078	215-228	(R)EGSVVMFGGVDHR(Y)			
1405.714	1405,6534	43,0948	216-228	(R)EGSVVMFGGVDHR(Y)	1Met-ex		
1733.952	1733,8838	39,4834	113-126	(FUFROHOSSTFRPTNK(T)			
1830.003	1829,9074	82 2487	346-361	(R)F888TETWILGDAFUR(V)			
1860.005	1850.875	80.873B	216-231	(R)EGSVVMFGGVDHRYYK(G)	1Med-coc		
1989.090	1998.9925	53,2689	232-248	(K)GELNWVPLIEEGDWSVR(M)			
2153.122	2153.0327	41 <i>A</i> 774	30-47	(KTILSGHNIMLNINFLIKEHGNR(L)	1P04		

Weaker matches were observed for PAG-17, PAG-16 and PAG-20

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